MICROSATELLITE PROBES FOR FINGERPRINTING OIL PALM CLONES

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Vegetative propagation of the oil palm by tissue culture was first described in the 1970s. Since then, the commercial advantage of tissue culture materials over seedlings has been well established. The initial problems or setbacks associated with oil palm tissue culture (for example, flowering abnormalities) have also been minimized by adopting low phytohormone protocols and high culling rates in the laboratory and nursery. This has led to renewed interest in *in vitro* production of oil palm clones on a large scale. The availability of simple, highly robust and low cost quality control probes for fingerprinting ortets to verify identity, monitor line uniformity and detect culture mix-ups will greatly enhance confidence in the commercial production of tissue culture materials.

QUALITY CONTROL PROCESS IN OIL PALM TISSUE CULTURE

Previously, the use of restriction fragment length polymorphic (RFLP) probes for quality control in the oil palm tissue culture process was described by Cheah *et al*. (1996). Although the process is effective, the drawbacks associated with using RFLP markers are that the analysis is expensive, time consuming and laborious.

To overcome these shortcomings, microsatellites, or simple sequence repeat (SSR) probes were developed for use in quality control for oil palm tissue culture. However, the SSR primers developed here do not address the issue of clonal abnormalities arising from somaclonal variation.

MICROSATELLITE PROBES

Microsatellites, or SSRs, are DNA sequences that consist of two (di), three (tri), four (tetra) or five (penta) nucleotide core units tandemly repeated. SSR loci are hypervariable in length, thus enabling their use for genetic identification. These genetic loci can be analysed by the polymerase chain reaction (PCR) using pairs of oligo nucleotide primers specific to the unique DNA sequences flanking the SSR.

NOVELTY AND ADVANTAGES OF USING SSR PROBES

The SSR primers described here were isolated from the oil palm genome. As such, these SSR probes will be more informative in their ability to distinguish genotypes compared with other marker systems such as random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) (Powell *et* al., 1996). The highly polymorphic nature of SSRs is of particular value in oil palm as the planting materials often involve narrowly adapted gene pools. SSR markers, which are PCR-based, have the advantage of reliability, reproducibility, discriminative ability and cost-effectiveness when compared to other marker systems (Smith *et al.*, 1997). Furthermore, since primer sequences are easy to share, this marker system is easily transferable from one laboratory to another.

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APPLICATION OF SSR PROBES IN THE TISSUE CULTURE PROCESS

Clonal Identification

In an attempt to show that monitoring of clonal fidelity during the tissue culture process is possible, DNA from ramets of four clones were compared with their respective ortet DNA. The results shown in *Figure 1* clearly demonstrate that there was no change in the SSR profiles of the ramets compared with those of their corresponding ortets. From the results, it is also clear that the SSR fingerprints can distinguish the four different ortets tested.

Monitoring Line Uniformity

SSR probes can be used to monitor line uniformity between and within the lines of a clone. A single oil palm clone with three lines was selected to demonstrate this, as shown in *Figure 2*.

Detection of Culture Mix-Up

Operator error causing cultures to be mixed is sometimes unavoidable given the large number of cultures that are handled daily in the tissue culture laboratory. These mistakes, however, need to be rectified in order to deliver a quality product to the customer. We tested the use of SSR analysis for this purpose. The SSR primers used were able to distinguish between the true ramets and *rogues* by comparing their SSR profiles with that of the ortet (*Figure 3*).

Recloning of Ramets

In order to recreate promising clones, several laboratories have resorted to resampling the ortet or recloning the ramets. Recloning the ramet offers the advantage that the number of plants available for sampling is multiplied. This is especially advantageous as the original ortet may no longer

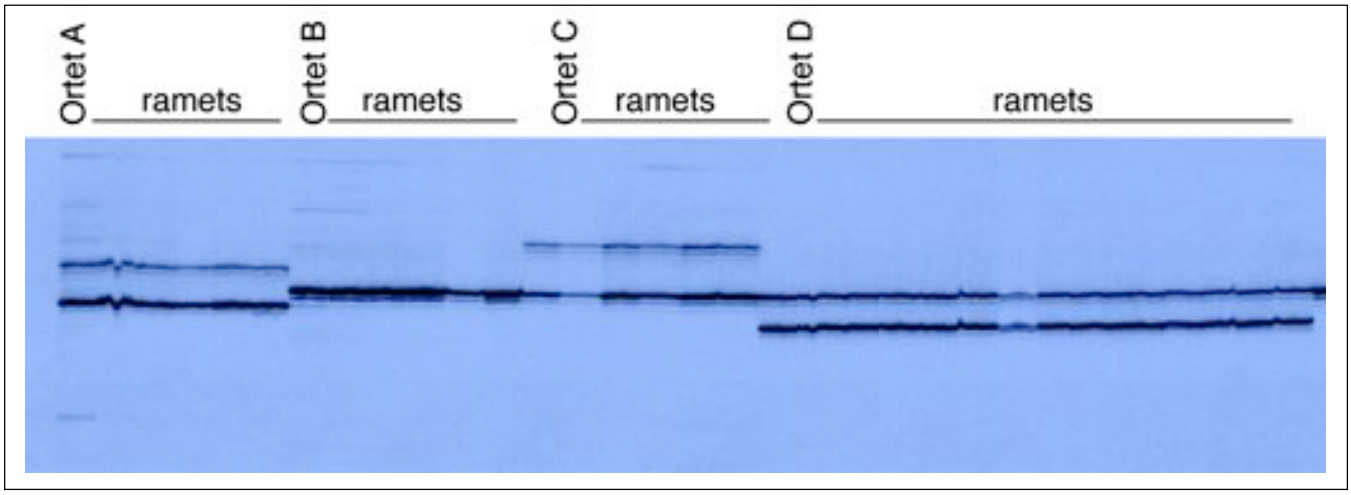


Figure 1. DNA fingerprinting of tissue culture clones using SSR analysis.

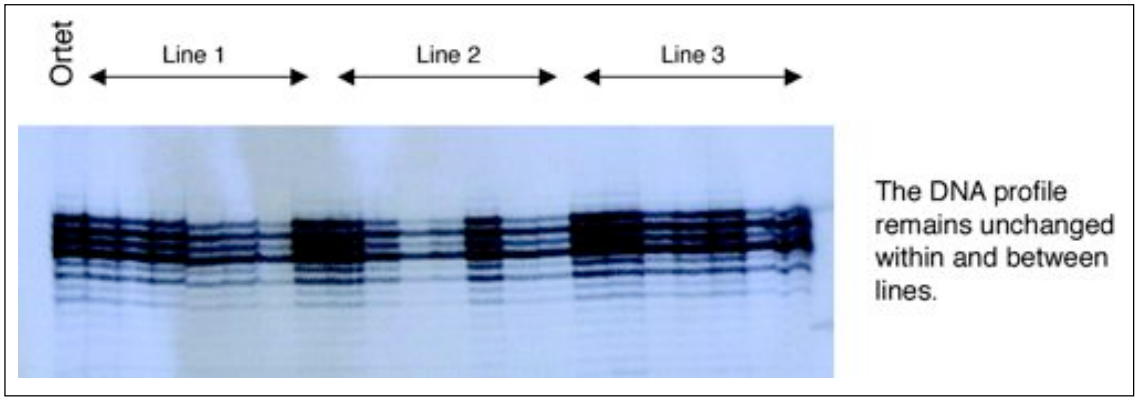
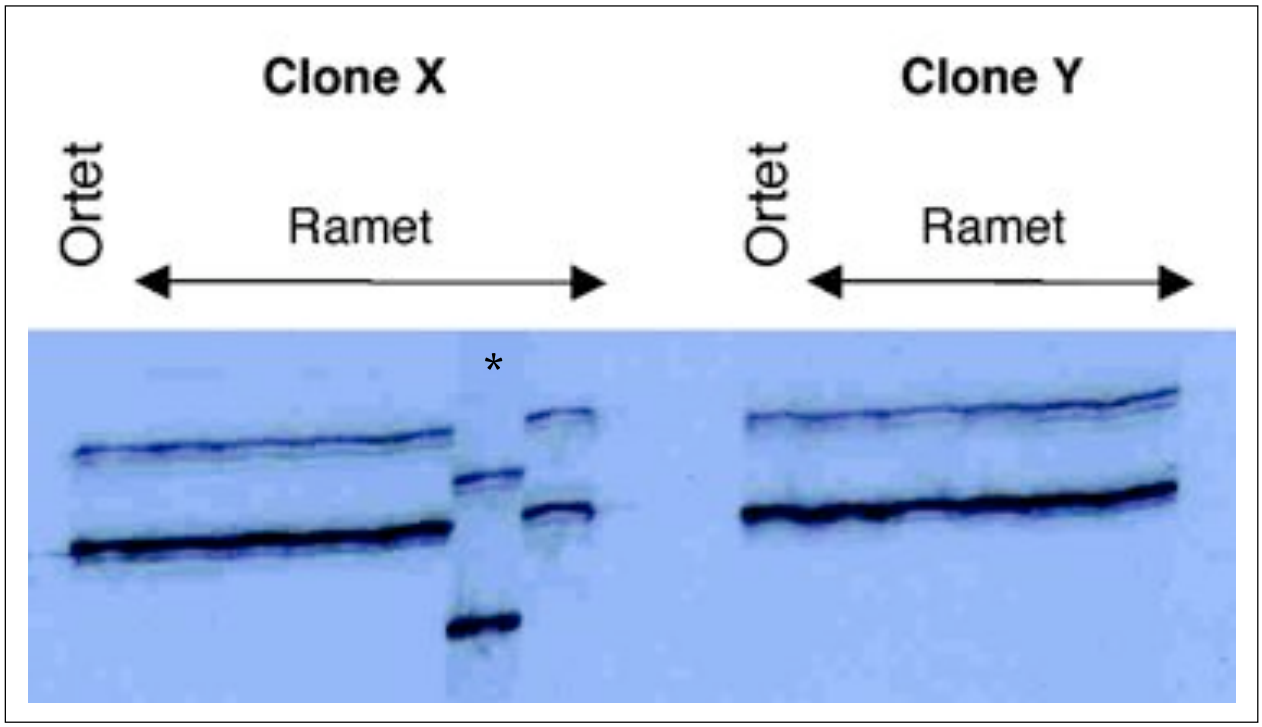


Figure 2. SSR fingerprinting for monitoring line uniformity.



Note: * Denotes a sample that did not originate from ortet X.

Figure 3. Detecting culture mix-up.

be available. In the sampling of ramets for recloning, it is imperative that the palm selected is authentically from the clone of choice. DNA fingerprinting will be useful for such verification.

PRICING AND ECONOMIC FEASIBILITY

The microsatellite fingerprinting process can be made available to interested parties either as a service or technology transfer.

Microsatellite Fingerprinting Service

Break-even analysis carried out for the above service at MPOB's laboratory at a capacity of 300 samples per year showed that the price charged must be at least RM 1200 for a set of three samples, with each sample tested with 10 microsatellite primer pairs.

Technology Transfer

It is envisaged that the technology can also be licensed to interested parties. A capital cost of about RM 1 million will be required to set up a properly functioning laboratory. Based on discounted cash flow (DCF) analysis, the project is viable for a laboratory with a capacity of 600 samples per year at a price of above RM 1800 per set of three samples.

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